

What is claimed is:

1. A method for detecting at least one target nucleic acid sequence in a sample comprising:

forming a ligation reaction composition comprising the sample and a ligation probe set for each target nucleic acid sequence, the probe set comprising

(a) at least one first probe, comprising a target-specific portion, a 5' primer-specific portion, wherein the 5' primer-specific portion comprises a sequence, and a first addressable portion located between the 5' primer-specific portion and the target-specific portion, wherein the first addressable portion comprises a sequence, and

(b) at least one second probe, comprising a target-specific portion, a 3' primer-specific portion, wherein the 3' primer-specific portion comprises a sequence, and a second addressable portion located between the 3' primer-specific portion and the target-specific portion, wherein the second addressable portion comprises a sequence,

wherein the probes in each set are suitable for ligation together when hybridized adjacent to one another on a complementary target nucleic acid sequence;

forming a test composition by subjecting the ligation reaction composition to at least one cycle of ligation, wherein adjacently hybridizing complementary probes are ligated to one another to form a ligation product comprising the 5' primer-specific portion, the first addressable portion, the target-specific portions, the second addressable portion, and the 3' primer-specific portion;

forming an amplification reaction composition comprising:

the test composition;

a polymerase;

a first labeled probe, wherein the first labeled probe has a first detectable signal value when it is not hybridized to a complementary sequence, and wherein the first labeled probe comprises the sequence of the first addressable portion or comprises a sequence complementary to the sequence of the first addressable portion;

a second labeled probe, wherein the second labeled probe has a first detectable signal value when it is not hybridized to a complementary sequence, and wherein the second labeled probe comprises the sequence of the second addressable portion or comprises a sequence complementary to the sequence of the second addressable portion;

and

at least one primer set, the primer set comprising (i) at least one first primer comprising the sequence of the 5' primer-specific portion of the ligation product, and (ii) at least one second primer comprising a sequence complementary to the sequence of the 3' primer-specific portion of the ligation product;

subjecting the amplification reaction composition to at least one amplification reaction; and

detecting a second detectable signal value from the first labeled probe and from the second labeled probe at least one of during and after the amplification reaction,

wherein a threshold difference between the first detectable signal value and the second detectable signal value of the first labeled probe and a threshold difference between the first detectable signal value and the second detectable signal

value of the second labeled probe indicates the presence of the target nucleic acid sequence; and wherein no threshold difference between the first detectable signal value and the second detectable signal value of the first labeled probe and no threshold difference between the first detectable signal value and the second detectable signal value of the second labeled probe indicates the absence of the target nucleic acid sequence.

2. The method of claim 1, wherein the labeled probe is a 5' nuclease probe.
3. The method of claim 2, wherein the 5' nuclease probe comprises at least one signal moiety and at least one quencher moiety.
4. The method of claim 3, wherein the least one signal moiety comprises at least one fluorescent moiety.
5. The method of claim 2, wherein the 5' nuclease probe comprises at least one signal moiety and at least one donor moiety.
6. The method of claim 1, wherein the labeled probe is a hybridization dependent probe.
7. The method of claim 6, wherein the hybridization dependent probe comprises at least one signal moiety and at least one quencher moiety.

8. The method of claim 7, wherein the least one signal moiety comprises at least one fluorescent moiety.

9. The method of claim 6, wherein the hybridization dependent probe comprises at least one signal moiety and at least one donor moiety.

10. The method of claim 1, wherein the labeled probe is a cleavable RNA probe.

11. The method of claim 10, wherein the cleavable RNA probe comprises at least one signal moiety and at least one quencher moiety.

12. The method of claim 11, wherein the least one signal moiety comprises at least one fluorescent moiety.

13. The method of claim 10, wherein the cleavable RNA probe comprises at least one signal moiety and at least one donor moiety.

14. The method of claim 1, wherein the at least one second probe further comprises a flap portion and a FEN cleavage position nucleotide, such that the target-specific portion of the at least one second probe is located between the FEN cleavage position nucleotide and the 3' primer-specific portion, and such that the FEN cleavage position nucleotide is located between the flap portion and the target-specific portion of the at least one second probe.

15. The method of claim 14, wherein the target-specific portion of the at least one first probe comprises a pivotal complement on an end of target-specific portion, such that the remainder of the target-specific portion is located between the 5' primer-specific portion and the pivotal complement, and wherein the FEN cleavage position nucleotide of the at least one second probe is the same as the pivotal complement of the at least one first probe.

16. The method of claim 15, wherein the ligation reaction composition further comprises flap endonuclease.

17. The method of claim 14, wherein the target-specific portion of the at least one first probe comprises (a) a pivotal complement that is at a penultimate position to an end of the target-specific portion and (b) a given nucleotide at that end of the target-specific portion, such that the remainder of the target-specific portion is located between the 5' primer-specific portion and the pivotal complement, and wherein the FEN cleavage position nucleotide of the at least one second probe is the same as the given nucleotide of the at least one first probe.

18. The method of claim 17, wherein the ligation reaction composition further comprises flap endonuclease.

19. The method of claim 14, wherein the target-specific portion of the at least one second probe comprises a pivotal complement at an end of the target-specific portion that is adjacent the FEN cleavage position nucleotide, and wherein the target-specific portion of the at least one first probe comprises a given nucleotide

at an end of the target-specific portion, such that the remainder of the target-specific portion is located between the 5' primer-specific portion and the given nucleotide, and wherein the FEN cleavage position nucleotide of the at least one second probe is the same as the given nucleotide of the at least one first probe.

20. The method of claim 19, wherein the ligation reaction composition further comprises flap endonuclease.

21. A method for detecting at least one target nucleic acid sequence in a sample comprising:

forming a ligation reaction composition comprising the sample and a ligation probe set for each target nucleic acid sequence, the probe set comprising (a) at least one first probe, comprising a target-specific portion and a 5' primer-specific portion, wherein the 5' primer-specific portion comprises a sequence, and (b) at least one second probe, comprising a target-specific portion and a 3' primer-specific portion, wherein the 3' primer-specific portion comprises a sequence;

wherein the probes in each set are suitable for ligation together when hybridized adjacent to one another on a complementary target nucleic acid sequence; wherein at least one of said at least one first probe and said at least one second probe further comprises (a) a first addressable portion located between the primer-specific portion and the target-specific portion, wherein the first addressable portion comprises a sequence, and (b) a second addressable portion located between the primer-specific portion and the target-specific portion, wherein the second addressable portion comprises a sequence;

forming a test composition by subjecting the ligation reaction composition to at least one cycle of ligation, wherein adjacently hybridizing complementary probes are ligated to one another to form a ligation product comprising the 5' primer-specific portion, the first addressable portion, the second addressable portion, the target-specific portions, and the 3' primer-specific portion;

forming an amplification reaction composition comprising:

the test composition;

a polymerase;

a first labeled probe, wherein the first labeled probe has a first detectable signal value when it is not hybridized to a complementary sequence, and wherein the first labeled probe comprises the sequence of the first addressable portion or comprises a sequence complementary to the sequence of the first addressable portion;

a second labeled probe, wherein the second labeled probe has a first detectable signal value when it is not hybridized to a complementary sequence, and wherein the second labeled probe comprises the sequence of the second addressable portion or comprises a sequence complementary to the sequence of the second addressable portion;

and

at least one primer set, the primer set comprising (i) at least one first primer comprising the sequence of the 5' primer-specific portion of the ligation product, and (ii) at least one second primer comprising a sequence complementary to the sequence of the 3' primer-specific portion of the ligation product;

subjecting the amplification reaction composition to at least one amplification reaction; and

detecting a second detectable signal value from the first labeled probe and from the second labeled probe at least one of during and after the amplification reaction,

wherein a threshold difference between the first detectable signal value and the second detectable signal value of the first labeled probe and a threshold difference between the first detectable signal value and the second detectable signal value of the second labeled probe indicates the presence of the target nucleic acid sequence; and wherein no threshold difference between the first detectable signal value and the second detectable signal value of the first labeled probe and no threshold difference between the first detectable signal value and the second detectable signal value of the second labeled probe indicates the absence of the target nucleic acid sequence.

22. A method for detecting at least one target nucleic acid sequence in a sample comprising:

(a) forming a reaction composition comprising:

the sample;

a ligation probe set for each target nucleic acid sequence, the probe set comprising (a) at least one first probe, comprising a target-specific portion and a 5' primer-specific portion, wherein the 5' primer-specific portion comprises a sequence and (b) at least one second probe, comprising a target-specific portion and a 3' primer-specific portion, wherein the 3' primer-specific portion comprises a sequence,

wherein the probes in each set are suitable for ligation together when hybridized adjacent to one another on a complementary target nucleic acid sequence, and wherein one probe in each probe set further comprises an addressable portion located between the primer-specific portion and the target-specific portion, wherein the addressable portion comprises a sequence;

a polymerase;

a labeled probe, wherein the labeled probe has a first detectable signal value when it is not hybridized to a complementary sequence, and wherein the labeled probe comprises the sequence of the addressable portion or comprises a sequence complementary to the sequence of the addressable portion; and

at least one primer set, the primer set comprising (i) at least one first primer comprising the sequence of the 5' primer-specific portion of the ligation product, and (ii) at least one second primer comprising a sequence complementary to the sequence of the 3' primer-specific portion of the ligation product;

(b) subjecting the reaction composition to at least one cycle of ligation, wherein adjacently hybridizing complementary probes are ligated to one another to form a ligation product comprising the 5' primer-specific portion, the target-specific portions, the addressable portion, and the 3' primer-specific portion;

(c) after the at least one cycle of ligation, subjecting the reaction composition to at least one amplification reaction; and

(d) detecting a second detectable signal value at least one of during and after the amplification reaction, wherein a threshold difference between the first detectable

signal value and the second detectable signal value indicates the presence of the target nucleic acid sequence, and wherein no threshold difference between the first detectable signal value and the second detectable signal value indicates the absence of the target nucleic acid sequence.

23. The method of claim 22, wherein the reaction composition further comprises a ligation reagent, wherein the ligation reagent activity is substantially destroyed prior to the at least one amplification reaction.

24. The method of claim 23, wherein the ligation reagent activity is substantially destroyed by subjecting the reaction composition to a temperature for a time period that substantially destroys the ligation reagent activity.

25. The method of claim 22, wherein the polymerase is substantially inactive during the at least one cycle of ligation and the polymerase is activated for the at least one amplification reaction.

26. The method of claim 25, wherein the polymerase is activated by subjecting the reaction composition to a temperature for a time period that activates the polymerase.

27. A method for detecting at least one target nucleic acid sequence in a sample comprising:

- (a) forming a reaction composition comprising:
 - the sample;

a ligation probe set for each target nucleic acid sequence, the probe set comprising:

(a) at least one first probe, comprising a target-specific portion, a 5' primer-specific portion, wherein the 5' primer-specific portion comprises a sequence, and a first addressable portion located between the 5' primer-specific portion and the target-specific portion, wherein the first addressable portion comprises a sequence, and

(b) at least one second probe, comprising a target-specific portion, a 3' primer-specific portion, wherein the 3' primer-specific portion comprises a sequence, and a second addressable portion located between the 3' primer-specific portion and the target-specific portion, wherein the second addressable portion comprises a sequence;

wherein the probes in each set are suitable for ligation together when hybridized adjacent to one another on a complementary target nucleic acid sequence;

a polymerase;

a first labeled probe, wherein the first labeled probe has a first detectable signal value when it is not hybridized to a complementary sequence, and wherein the first labeled probe comprises the sequence of the first addressable portion or comprises a sequence complementary to the sequence of the first addressable portion;

a second labeled probe, wherein the second labeled probe has a first detectable signal value when it is not hybridized to a complementary sequence, and wherein the second labeled probe comprises the sequence of

the second addressable portion or comprises a sequence complementary to the sequence of the second addressable portion; and

at least one primer set, the primer set comprising (i) at least one first primer comprising the sequence of the 5' primer-specific portion of the ligation product, and (ii) at least one second primer comprising a sequence complementary to the sequence of the 3' primer-specific portion of the ligation product;

(b) subjecting the reaction composition to at least one cycle of ligation, wherein adjacently hybridizing complementary probes are ligated to one another to form a ligation product comprising the 5' primer-specific portion, the first addressable portion, the target-specific portions, the second addressable portion, and the 3' primer-specific portion;

(c) after the at least one cycle of ligation, subjecting the reaction composition to at least one amplification reaction; and

(d) detecting a second detectable signal value from the first labeled probe and from the second labeled probe at least one of during and after the amplification reaction,

wherein a threshold difference between the first detectable signal value and the second detectable signal value of the first labeled probe and a threshold difference between the first detectable signal value and the second detectable signal value of the second labeled probe indicates the presence of the target nucleic acid sequence; and wherein no threshold difference between the first detectable signal value and the second detectable signal value of the first labeled probe and no threshold difference between the first detectable signal value and the second

detectable signal value of the second labeled probe indicates the absence of the target nucleic acid sequence.

28. A method for detecting at least one target nucleic acid sequence in a sample comprising:

(a) forming a reaction composition comprising:

the sample;

a ligation probe set for each target nucleic acid sequence, the probe set comprising (a) at least one first probe, comprising a target-specific portion and a 5' primer-specific portion, wherein the 5' primer-specific portion comprises a sequence, and (b) at least one second probe, comprising a target-specific portion and a 3' primer-specific portion, wherein the 3' primer-specific portion comprises a sequence;

wherein the probes in each set are suitable for ligation together when hybridized adjacent to one another on a complementary target nucleic acid sequence; wherein at least one of said at least one first probe and said at least one second probe further comprises (a) a first addressable portion located between the primer-specific portion and the target-specific portion, wherein the first addressable portion comprises a sequence, and (b) a second addressable portion located between the primer-specific portion and the target-specific portion, wherein the second addressable portion comprises a sequence;

a polymerase;

a first labeled probe, wherein the first labeled probe has a first detectable signal value when it is not hybridized to a complementary sequence, and wherein the first labeled probe comprises the sequence of the

first addressable portion or comprises a sequence complementary to the sequence of the first addressable portion;

a second labeled probe, wherein the second labeled probe has a first detectable signal value when it is not hybridized to a complementary sequence, and wherein the second labeled probe comprises the sequence of the second addressable portion or comprises a sequence complementary to the sequence of the second addressable portion; and

at least one primer set, the primer set comprising (i) at least one first primer comprising the sequence of the 5' primer-specific portion of the ligation product, and (ii) at least one second primer comprising a sequence complementary to the sequence of the 3' primer-specific portion of the ligation product;

(b) subjecting the reaction composition to at least one cycle of ligation, wherein adjacently hybridizing complementary probes are ligated to one another to form a ligation product comprising the 5' primer-specific portion, the first addressable portion, the target-specific portions, the second addressable portion, and the 3' primer-specific portion;

(c) after the at least one cycle of ligation, subjecting the reaction composition to at least one amplification reaction; and

(d) detecting a second detectable signal value from the first labeled probe and from the second labeled probe at least one of during and after the amplification reaction,

wherein a threshold difference between the first detectable signal value and the second detectable signal value of the first labeled probe and a threshold difference between the first detectable signal value and the second detectable signal

value of the second labeled probe indicates the presence of the target nucleic acid sequence; and wherein no threshold difference between the first detectable signal value and the second detectable signal value of the first labeled probe and no threshold difference between the first detectable signal value and the second detectable signal value of the second labeled probe indicates the absence of the target nucleic acid sequence.

29. A method of making a library of probes comprising:

synthesizing a first library of 4^X probes each comprising a primer-specific portion, a target-specific portion X nucleotides in length, and a first addressable portion located between the primer-specific portion and the target-specific portion, wherein each of the 4^X probes of the first library of 4^X probes comprises a different target-specific portion, wherein X is 4 to 8.

30. The method of claim 29, further comprising:

synthesizing a second library of 4^X probes each comprising a primer-specific portion, a target-specific portion X nucleotides in length, and a second addressable portion located between the primer-specific portion and the target-specific portion, wherein each of the 4^X probes of the second library of 4^X probes comprises a different target-specific portion, and wherein the second addressable portion has a different sequence than the first addressable portion, wherein X is 4 to 8.

31. A method of selecting a probe comprising:

selecting from a first library of probes a probe that comprises a target-specific portion that is complementary to a desired portion of X nucleotides of a target nucleic acid sequence, wherein X is 4 to 8;

wherein the first library of probes comprises 4^X probes that each comprise a primer-specific portion, a target-specific portion X nucleotides in length, and a first addressable portion located between the primer-specific portion and the target-specific portion, wherein each of the 4^X probes comprises a different target-specific portion.

32. A first library of 4^X probes each comprising a primer-specific portion, a target-specific portion X nucleotides in length, and a first addressable portion located between the primer-specific portion and the target-specific portion, wherein each of the 4^X probes of the first library of 4^X probes comprises a different target-specific portion, wherein X is 4 to 8.

33. A method of making a library of $(4^{(X-1)} \text{ multiplied by } 6)$ pairs of probes, comprising synthesizing a library of $(4^{(X-1)} \text{ multiplied by } 6)$ pairs of probes;

wherein one probe of each pair comprises a primer-specific portion, a target-specific portion comprising a sequence of X nucleotides, and a first addressable portion located between the primer-specific portion and the target-specific portion,

wherein the other probe of each pair comprises a primer-specific portion, a target-specific portion comprising a sequence of X nucleotides, and a second addressable portion located between the primer-specific portion and the target-specific portion,

wherein the sequence of X nucleotides of the target-specific portion of each probe in a pair of probes is identical except for one nucleotide difference;

wherein each of the $(4^{(X-1)} \text{ multiplied by } 6)$ pairs of probes can be used to determine whether a target nucleic acid sequence comprising X nucleotides has one of two possible nucleic acid sequences, wherein the two possible nucleic acid sequences differ by one nucleotide at a single position, and wherein at least one separate pair of probes of the library is provided for each separate possible one nucleotide difference at one position in a target nucleic acid comprising X nucleotides; and

wherein X is 4 to 8.

34. A library of $(4^{(X-1)} \text{ multiplied by } 6)$ pairs of probes;

wherein one probe of each pair comprises a primer-specific portion, a target-specific portion comprising a sequence of X nucleotides, and a first addressable portion located between the primer-specific portion and the target-specific portion,

wherein the other probe of each pair comprises a primer-specific portion, a target-specific portion comprising a sequence of X nucleotides, and a second addressable portion located between the primer-specific portion and the target-specific portion,

wherein the sequence of X nucleotides of the target-specific portion of each probe in a pair of probes is identical except for one nucleotide difference;

wherein each of the $(4^{(X-1)} \text{ multiplied by } 6)$ pairs of probes can be used to determine whether a target nucleic acid sequence comprising X nucleotides has one of two possible nucleic acid sequences, wherein the two possible nucleic acid sequences differ by one nucleotide at a single position, and wherein at least one

separate pair of probes of the library is provided for each separate possible one nucleotide difference at one position in a target nucleic acid comprising X nucleotides; and

wherein X is 4 to 8.

35. A kit for detecting at least one target nucleic acid sequence in a sample comprising:

a ligation probe set for each target nucleic acid sequence, the probe set comprising

(a) at least one first probe, comprising a target-specific portion, a 5' primer-specific portion, wherein the 5' primer-specific portion comprises a sequence, and a first addressable portion located between the 5' primer-specific portion and the target-specific portion, wherein the first addressable portion comprises a sequence, and

(b) at least one second probe, comprising a target-specific portion, a 3' primer-specific portion, wherein the 3' primer-specific portion comprises a sequence, and a second addressable portion located between the 3' primer-specific portion and the target-specific portion, wherein the second addressable portion comprises a sequence,

wherein the probes in each set are suitable for ligation together when hybridized adjacent to one another on a complementary target nucleic acid sequence;

a first labeled probe comprising the addressable sequence of the first addressable portion or comprising a sequence complementary to the sequence of the first addressable portion; and

a second labeled probe comprising the sequence of the second addressable portion or comprising a sequence complementary to the sequence of the second addressable portion.

36. The kit of claim 35,

wherein the first labeled probe has a first detectable signal value when it is not hybridized to a complementary sequence, and a second detectable signal value of the first labeled probe can be detected at least one of during and after an amplification reaction; and

wherein the second labeled probe has a first detectable signal value when it is not hybridized to a complementary sequence, and a second detectable signal value of the second labeled probe can be detected at least one of during and after an amplification reaction; and

wherein a threshold difference between the first detectable signal value and the second detectable signal value of the first labeled probe and a threshold difference between the first detectable signal value and the second detectable signal value of the second labeled probe indicates the presence of the target nucleic acid sequence; and wherein no threshold difference between the first detectable signal value and the second detectable signal value of the first labeled probe and no threshold difference between the first detectable signal value and the second detectable signal value of the second labeled probe indicates the absence of the target nucleic acid sequence.

37. The kit of claim 35, further comprising at least one primer set comprising (i) at least one first primer comprising the sequence of the 5' primer-

specific portion of the at least one first probe, and (ii) at least one second primer comprising a sequence complementary to the sequence of the 3' primer-specific portion of the at least one second probe.

38. A kit for detecting at least one target nucleic acid sequence in a sample comprising:

a ligation probe set for each target nucleic acid sequence, the probe set comprising

(a) at least one first probe, comprising a target-specific portion and a 5' primer-specific portion, wherein the 5' primer-specific portion comprises a sequence, and (b) at least one second probe, comprising a target-specific portion and a 3' primer-specific portion, wherein the 3' primer-specific portion comprises a sequence,

wherein the probes in each set are suitable for ligation together when hybridized adjacent to one another on a complementary target nucleic acid sequence; wherein at least one of said at least one first probe and said at least one second probe further comprises (a) a first addressable portion located between the primer-specific portion and the target-specific portion, wherein the first addressable portion comprises a sequence, and (b) a second addressable portion located between the primer-specific portion and the target-specific portion, wherein the second addressable portion comprises a sequence;

a first labeled probe comprising the addressable sequence of the first addressable portion or comprising a sequence complementary to the sequence of the first addressable portion; and

a second labeled probe comprising the sequence of the second addressable portion or comprising a sequence complementary to the sequence of the second addressable portion.

39. The kit of claim 38,

wherein the first labeled probe has a first detectable signal value when it is not hybridized to a complementary sequence, and a second detectable signal value of the first labeled probe can be detected at least one of during and after an amplification reaction; and

wherein the second labeled probe has a first detectable signal value when it is not hybridized to a complementary sequence, and a second detectable signal value of the second labeled probe can be detected at least one of during and after an amplification reaction; and

wherein a threshold difference between the first detectable signal value and the second detectable signal value of the first labeled probe and a threshold difference between the first detectable signal value and the second detectable signal value of the second labeled probe indicates the presence of the target nucleic acid sequence; and wherein no threshold difference between the first detectable signal value and the second detectable signal value of the first labeled probe and no threshold difference between the first detectable signal value and the second detectable signal value of the second labeled probe indicates the absence of the target nucleic acid sequence.

40. The kit of claim 38, further comprising at least one primer set comprising (i) at least one first primer comprising the sequence of the 5' primer-

specific portion of the at least one first probe, and (ii) at least one second primer comprising a sequence complementary to the sequence of the 3' primer-specific portion of the at least one second probe.

41. A method for detecting at least one target nucleic acid sequence in a sample comprising:

forming a ligation reaction composition comprising the sample and a ligation probe set for each target nucleic acid sequence, the probe set comprising (a) at least one first probe, comprising a target-specific portion and a 5' primer-specific portion, wherein the 5' primer-specific portion comprises a sequence, and (b) at least one second probe, comprising a target-specific portion and a 3' primer-specific portion, wherein the 3' primer-specific portion comprises a sequence, and wherein a minor groove binder is attached to said second probe,

wherein the probes in each set are suitable for ligation together when hybridized adjacent to one another on a complementary target nucleic acid sequence;

forming a test composition by subjecting the ligation reaction composition to at least one cycle of ligation, wherein adjacently hybridizing complementary probes are ligated to one another to form a ligation product comprising the 5' primer-specific portion, the target-specific portions, and the 3' primer-specific portion;

forming an amplification reaction composition comprising:

the test composition;

a polymerase; and

at least one primer set, the primer set comprising (i) at least one first primer comprising the sequence of the 5' primer-specific portion of the

ligation product, and (ii) at least one second primer comprising a sequence complementary to the sequence of the 3' primer-specific portion of the ligation product;

subjecting the amplification reaction composition to at least one amplification reaction; and

detecting the presence or absence of the at least one target nucleic acid by detecting the presence or absence of the ligation product.

42. The method of claim 41, wherein a minor groove binder is attached to the first primer.

43. A method for detecting at least one target nucleic acid sequence in a sample comprising:

forming a reaction composition comprising

the sample,

a ligation probe set for each target nucleic acid sequence, the probe set comprising (a) at least one first probe, comprising a target-specific portion and a 5' primer-specific portion, wherein the 5' primer-specific portion comprises a sequence, and (b) at least one second probe, comprising a target-specific portion and a 3' primer-specific portion, wherein the 3' primer-specific portion comprises a sequence, and wherein a minor groove binder is attached to said second probe,

wherein the probes in each set are suitable for ligation together when hybridized adjacent to one another on a complementary target nucleic acid sequence;

a polymerase; and

at least one primer set, the primer set comprising (i) at least one first primer comprising the sequence of the 5' primer-specific portion of the ligation product, and (ii) at least one second primer comprising a sequence complementary to the sequence of the 3' primer-specific portion of the ligation product;

subjecting the reaction composition to at least one cycle of ligation, wherein adjacently hybridizing complementary probes are ligated to one another to form a ligation product comprising the 5' primer-specific portion, the target-specific portions, and the 3' primer-specific portion;

after the at least one cycle of ligation, subjecting the reaction composition to at least one amplification reaction; and

detecting the presence or absence of the at least one target nucleic acid by detecting the presence or absence of the ligation product.

44. The method of claim 43, wherein a minor groove binder is attached to the first primer.